THE METHOD OF PROCEDURE IN THE ANALYSIS OF HEREDITY

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WHATEVER the conception we may have of the essential nature of the activities of living things, it must be agreed that as time goes on more and more of them can be pictured in terms of demonstrable mechanical models. I shall leave to others the questions: Why does the biologist get any satisfaction out of such constructions and why does he not rather busy himself with the determination of absolute values?

The first step toward a satisfactory basis for the understanding of the nature of the transmission of hereditary qualities was the proof that organisms as we know them are never derived from non-living things. They are always separated parts of a parental organism something like themselves. This simple proposition was not demonstrated until the last century. From the earliest times it was commonly believed that certain animals at least can be generated from non-living material by the action of external agents. If such a generation is possible it seriously affects our notions regarding the transmission of hereditary qualities. As far as we now know every organism starts as part of a preexisting organism. Our question then is the manner in which this part of the parental body carries the qualities of the future adult individual.

That the qualities of the separated part or germ cell are really of great importance in the development of the new individual, as compared with environmental forces, may be readily demonstrated by placing a fish egg and a frog's egg side by side in a dish of water. The surroundings are the same for both and yet one develops into a fish and the other into a frog. No environmental differences can produce effects at all comparable with these. The relation of these biological facts to certain sociological theories is obvious. The primary differences between human beings as between other organisms are due to hereditary factors and not to environmental factors.

Some have claimed that one egg develops into a fish because it has a non-material force or entelechy which wants it to develop into a fish while the other egg has an entelechy which wants to it develop into a frog. If it were impossible to

make out any units of a lower order than the eggs, if they were the ultimate particles with no visible differences between them, in despair of any other explanation one might postulate that differences in their activities are due to such non-material forces. This is no more than the physicist does with his ultimate particles, though he does not often admit it. On the other hand, more physicists than biologists are anxious to prove that the smallest known particles of organisms have souls.

However, since the egg is not an ultimate particle and it is possible to make out something of the structure and activities of its various parts, the biologist tries to picture to himself the way in which these parts are related to the adult characteristics. He tries to determine how they would act if they were large enough to be handled.

Preformation and Epigenesis

From the time of the earliest philosophers some have denied the problem of individual development by claiming that the egg contains the parts of the adult in miniature and that development is merely an enlargement of these parts. On the other hand, some have denied the presence of structure within the egg and have claimed that development starts with no structure and gradually works toward the complexity of the adult. It will not be worth while to follow here the early history of this controversy between the preformationists or evolutionists, as they were called, and the opposing school of the epigeneticists. It may suffice to say that opinion alternated from one extreme to the other. In the seventeenth century, when the first compound microscopes were used to examine eggs and spermatozoa, the observers were so convinced that the human body was present in miniature that they promptly found it there and published their findings in elaborate drawings of the little mannikin with its limbs nicely folded up like the petals in a flower bud. Those of us who have had to do with students in biological laboratories recognize that people have not changed in this regard during the centuries. It is still very easy to see what you are looking for and still very hard to see things that do not fit into your preconceived notion.

With the construction of better microscopes it was soon made evident that the little mannikins do not exist and there was an early swing of opinion to the opposite extreme. Practically all biologists became epigeneticists, claiming that the egg

is a homogeneous protoplasm in which the adult structures are gradually developed.

Since the middle of the last century, however, there has been a gradual return from this extreme position. Improvement in microscope lenses has made possible a rapid advance in the knowledge of the structure of organisms. It has been shown that organisms are made up of smaller units, the cells, that the ovum and spermatozoon are such units and that all the numerous cells of the adult body are derived from the subdivision of a single cell, the fertilized ovum. Furthermore, it was shown that there is a complicated but very definite mechanism within all cells. Experimental work has demonstrated a specific relation between this mechanism within the egg and the adult structure. The general trend, therefore, is toward a modified preformation. The parts of the adult are not entirely unrepresented in the egg, as in the view of the extreme epigeneticists, nor are they represented by exact miniatures, as in the view of the extreme preformationists or evolutionists. Instead there is a recognition of a definite, specific relation between certain structures and activities in the egg and certain other structures and activities in the adult.

Nucleus and Cytoplasm

The first step in the analysis is the recognition of the difference in function between the cytoplasm and the nucleus of the egg. Within the egg as in every cell two portions can be recognized, a central body called the nucleus surrounded by the remaining substance called the cytoplasm. During the last fifty years a large amount of evidence has been collected which proves that the nucleus and cytoplasm have different functions not only in the ordinary life of the cell, but also in their relations to the transmission of hereditary qualities.

Histological studies have shown that the visible differences in the different tissues of an organism are almost wholly if not wholly in their cytoplasms. The essential differences that we make out between the different cells of the body have to do with cytoplasmic structures. Thus the muscle fibrils, the nerve fibers, the pigment granules and similar modifications in the tissues are all cytoplasmic. There is reason to believe, however, that the nucleus has some causal connection with their appearance.

It has been demonstrated that while both nucleus and cytoplasm must be present in order that development may

occur, they are by no means of equal value in the process. In certain eggs if the nucleus is left undisturbed the greater part of the cytoplasm may be removed without affecting the development of a complete normal individual, and it does not matter what part is so removed. Thus while the nucleus alone can not develop, a small amount of cytoplasm from any location is sufficient to cause it to do so. On the other hand, when the cytoplasm remains intact not only the removal of a part of the nucleus, but even a disarrangement of its materials is sufficient to prevent the development of a normal individual.

Another point in the evidence is the fact that, while on the whole the female and male parents contribute equally toward the qualities of the child, the cytoplasmic contribution of the spermatozoon is negligible in amount. On the other hand, the amount of nuclear material furnished by egg and spermatozoon is essentially equal in amount and, as we shall see, this similarity applies to the details of nuclear structure. Furthermore, the nucleus and not the cytoplasm contains a mechanism in agreement with the facts of experimental breeding.

THE CHROMOSOMES

It is in the nucleus then that we are bound to seek this further mechanism of heredity. Our evidence for such a conclusion has been accumulating very rapidly during recent years, and it is not possible to do more than give some of the striking points.

A detailed microscopical study has shown that there is within the nucleus a material, called chromatin because of its affinity for certain dyes, which behaves in a remarkable manner during each cell division. This material is present in the period between cell divisions in the form of granules. Preceding a cell division, these granules arrange themselves in a row or rows, producing a thread or threads of granules which soon break up into definite segments or chromosomes. These chromosomes are perfectly definite bodies, always the same in number in any species of animal, always breaking up into their constituent granules between cell divisions and always being built up again at every cell division from the egg to the adult. A beautiful structure somewhat resembling the diagrams of a magnetic field is then developed, with two poles, the centrosomes, and with radiations extending in all directions from them. The rays passing from one pole to the other constitute what is known as the spindle. The chromosomes

arrange themselves in a transverse plane at the center of this spindle, each one splits longitudinally, and the two halves travel to opposite poles. The cell then constricts at its equator and two daughter cells are produced each containing a longitudinal half of each of the chromosomes. The whole elaborate mechanism has this one important function of bringing about the exact distribution of chromatin material, so that each daughter cell gets not only the same total amount as the other, but also exactly the same amount of each part of each chromosome. By the repetition of this process every cell in the adult body finally has exactly the same chromosomal complement as every other cell.

It follows from this fact that, on the chromosomal hypothesis, every cell contains a complete set of developmental determiners. Then, why do some cells form muscles, others nerves, still others connective tissue and so on? Weismann's theory involved the assumption that the cell divisions were actually qualitative and that the different cells of the body obtain different complements of chromatin. As stated there is no observational basis for such a conclusion. We are therefore forced to the hypothesis that each cell has all of the materials and the question of why it uses some and not others remains to be solved by other means. The discussion of this problem, however, can not be undertaken here.

There is an interesting modification of this process of equal distribution of chromosomes, during the last two divisions of the germ-cells, those immediately preceding the time when they are ready for fertilization. During the early divisions of the germ cells there is no essential difference between them and other cells, but at one of the last two divisions instead of the ordinary procedure of a longitudinal splitting of each of the chromosomes which would insure the original number in each of the daughter cells, there is no splitting at all. Two whole chromosomes come to lie side by side in the equator of the spindle and each of the daughter or mature germ cells gets a half of the total number. There is thus a reduction to one half of the original number of chromosomes. This takes place in both the male and the female germ cells. For a reason to be mentioned presently, it is customary to speak of this reduced number as n and of the number in the division of ordinary cells as $2n$. It is obvious since spermatozoon and mature ovum each contain n chromosomes that when they unite in fertilization the $2n$ number is restored.

There are a number of other interesting points in connection with this reduction in the number of chromosomes. It

has been shown that a mature unfertilized egg may be caused to start development in other ways than by union with a spermatozoon. The method is not important, since the same result may be brought about by a great many different kinds of agents, as chemical change in the medium, osmotic change, rapid change in temperature, pricking with a needle or even by shaking. The cells of individuals developed in this way have only the n set of chromosomes from the ovum, yet they produce complete individuals. Likewise, a small piece of the cytoplasm of the egg without any nucleus may be entered by a spermatozoon, and the nucleus of the resulting fusion contains only the n chromosomes of the male. Yet a whole individual results again. It is clear, therefore, that if the chromosomes contain the essential factors in the development of the characters of the individual, the egg contains a complete set of such factors and the spermatozoon contains another complete set. The fertilized egg and all of the cells of the body derived from it must therefore contain a double set.

This is in agreement with the facts obtained by experimental breeding as first made out by Mendel in 1866. Since its rediscovery in 1900 the principle involved in the so-called Mendelian inheritance has been shown to be a general one. A great many hundreds of characters in both animals and plants have been shown to follow it.

The essential point in these phenomena, as pointed out by Mendel before there was any knowledge of a chromosomal mechanism, is that the body of an organism contains a double set of factors, one or more pairs for each of its characters. Any character then is dependent upon the presence of at least two factors, one derived from the male and the other from the female, and these two factors must separate again when the sex cells are produced. Each sex cell then can have only one member of the original pair. Of course, this fact can only be demonstrated when the factor coming from the male is different from that coming from the female, as in hybridization. If in such a case we call the factor coming from one parent A and that from the other parent a, then the resulting individual will have the constitution Aa. When it produces sex cells half of them must carry A alone and the other half a alone in order to get the proportions obtained in experimental breeding which are one fourth with AA, one half with Aa and one fourth with aa. As Mendel observed, the A and the a show no contamination as a result of their intimate association within the same body. They are as pure as they were in the original parents.

Mendel showed further that in case there is present in the

same mating another pair of characters due to another pair of factors as *B* and *b*, their distribution is independent of the distribution of *A* and *a*. In the second hybrid generation there is thus a combination of characters which is the one to be expected on the view of independent assortment of the two pairs of factors.

It happens that the behavior of the chromosomes is such as to furnish an ideal mechanism for this distribution of factors. If we place the hypothetical factors for the Mendelian characters in the chromosomes of our model they are distributed in exactly the proper way to give rise to the numerical proportions of Mendel's law.

Differences in the factors contained do not, however, as a rule cause visible differences in the chromosomes which carry them. There is only a single demonstrated case of such a difference and that is in the inheritance of sex. This case is therefore of the greatest interest. In order to make the explanation as simple as possible, I shall take only one of the kinds of differences that have been made out. In a great many animals there is an exception to the rule that $2n$ chromosomes are present in the cells of the body. Instead there are $2n-1$ chromosomes in the cells of the males, while the females have the ordinary number $2n$. When mature eggs are being produced there is the ordinary reduction in the number of chromosomes to one half and all obtain n chromosomes. In the spermatozoa there can not be such an equality because $2n-1$ is an odd number. Accordingly, when the chromosomes pair off in preparation for the maturation divisions, one is left without a mate. One of the daughter cells obtains n chromosomes and the other only $n-1$. Accordingly, half of the spermatozoa are of one kind and half of the other. It follows that since all eggs are alike in having n chromosomes the result of random or non-selective mating gives half of the individuals with $2n$ chromosomes, or the number in the female body cells, and half with $2n-1$ the number in the male body cells.

Another result follows from this consideration. If the factors for other characters than sex are located in these chromosomes they should be distributed according to a scheme differing from that of other characters. This follows from the fact that in the case of such characters half of the spermatozoa should lack entirely any factor for them. Numerous such sex-linked characters are known.

If the chromosomal hypothesis is correct, it follows further that the number of independently heritable characters as far as random distribution is concerned should be limited to the

number of pairs of chromosomes in the species in question. If the factors for two characters are located in the same chromosome they should go together or be linked according to the technical expression. Such linkage has been demonstrated frequently. Furthermore, there is no known case in which there are more independent groups of linked characters than there are pairs of chromosomes. The form in which the heredity of the greatest number of characters has been worked out is the fruit fly *Drosophila* with over 200 to its credit. There are only four pairs of chromosomes and correspondingly the characters are linked in inheritance in four groups. Furthermore, one of the four pairs of chromosomes is very small and correspondingly one of the linked groups of characters is much smaller than the others.

This striking mass of evidence from normal inheritance is confirmed by the experiments with abnormal distribution of chromosomes. The two cases I shall choose in illustration approach the problem from opposite sides.

Boveri produced an abnormal distribution of the chromosomes during the first cleavage of the egg by inducing two spermatozoa to enter the egg at once. He then separated the daughter cells. This was in the sea-urchin egg, a form in which under normal conditions separated cells produce complete individuals. Only a certain percentage of these daughter cells had full sets of chromosomes. The same percentage developed into complete individuals.

Bridges attacked the problem from the other side. In some of his fruit-fly material the inheritance of the characters did not follow the ordinary Mendelian formula. He figured out the kind of chromosomal abnormality that would yield such a result. He decided that the breeding data would follow if, in the maturation of the egg cell, the members of the pair of chromosomes involved did not separate as in normal reduction, but went to the same pole, leaving one of the daughter cells with both members of the pair and the other without any. An examination of these cells made after the formulation of this explanation showed that such an abnormal separation had actually occurred.

These experiments with irregular distribution clinch the argument that the chromosomes are the bearers of factors having to do with the appearance of characters.

THE CHROMOMERES

Within the last few years an extension of our knowledge has shown that the chromosomes can not be considered as the

elementary units in the transmission of hereditary qualities. An analysis of the differences in value between different parts of individual chromosomes is therefore being made.

The possibility of such an analysis was already indicated by the microscopical observations previously mentioned, which showed that the chromosomes are themselves made up of rows of granules. These individual granules are known as chromomeres. It will be recalled that when the chromosomes are formed during cell division each one is made up by the coming together of the granules present in the resting stage of the cell. A large number of cytological observations have made it seem probable that when a chromosome breaks up into its constituent granules or chromomeres at the end of a cell division, these granules do not mix up with others in the nucleus, but occupy a definite region in it. This is made out especially well in certain lobed nuclei in which the separate regions belonging to the individual chromosomes can be definitely mapped out. It is probable therefore that the same granules form homologous chromosomes in succeeding cell generations.

It follows also from the nature of the division of a chromosome that when it splits longitudinally into two equal parts, each granule or chromomere is also split into two equal parts and, therefore, each daughter cell obtains not only a half of each of the chromosomes, but also a half of each of the constituent granules. Each cell of the completed body, therefore, has its equal share of each of the minute chromatin granules present in the egg.

Supposing each granule or chromomere to represent a different kind of material, each cell of the organism has a complete set of materials. All the cells are then qualitatively alike in this respect. The quantitative relations are restored between succeeding cell divisions by growth, as each chromomere is able to build up new material like itself.

It is probable that here in the chromomeres are elements in the mechanism of heredity of a lower order than the chromosomes. If the chromomeres of a chromosome always stick together or if the linkage of characters within a group is never broken there is no way of testing such a hypothesis. Fortunately we have evidence from both sides of such a breaking of linkage.

From the side of experimental breeding, evidence has accumulated that while, according to the hypothesis that the chromosomes are indivisible units, linked characters should stick together, they do not always do so. This breaking of the linkage was subjected to careful study, particularly in the

fruit fly, *Drosophila,* and it was shown that the breaking never takes place in the formation of the spermatozoa but only in the formation of the eggs. Furthermore, taking a linked group such as that which is found in the same chromosome as the sex-determining factor, the percentage of breaking of the linkage between any two factors is fairly constant. If the percentages between characters *a* and *b* and between *b* and *c* are known, that between *a* and *c* is either the sum or the difference of the others. The fifty or so characters in this linked group all fit into this linear arrangement. A line with the factors located upon it can be drawn, in which the distance between any two points, representing the location of factors, corresponds to the percentage of breaking of linkage between those two points. Such diagrams have been carefully constructed. For instance, the percentage of separation of the characters yellow body and white eye is 1.2, of white eye and bifid wing 3.5, and of yellow body and bifid wing 4.7, or the sum of the other two.

As stated, this linear arrangement, in which the distance between any two factors is proportional to the percentage of separation of the characters, is fairly consistent but not wholly so. There is a tendency for the high values calculated from the sums of two components to be somewhat higher than the actual ones. The suggested explanations will be given later.

It is perfectly natural to suppose that this linear arrangement on the basis of percentage of separation of the factors in breeding may represent an actual linear arrangement within the chromosomes. This necessitates the postulate that the chromatin granules as they pass from the resting stage preceding a cell division always arrange themselves in the same definite, fixed order when they form a chromosome. It is only recently that there has been any cytological evidence bearing on this point.

Assuming that the granules actually do lie in a fixed order, to explain the facts of the breaking of linkage it is necessary to discover a mechanism by which the granules of one chromosome may be exchanged for those of its mate as the two lie side by side at the beginning of the maturation divisions of the egg.

It will be remembered that the two chromosomes which lie side by side in this manner come from separate parents. It was supposed that they always separate as units, but it has been known for some years that they frequently twist around each other, and may indeed seem to fuse at the point where one

crosses the other. It was supposed until recently that when the members of the pair separate to travel toward opposite poles they have undergone no exchange of material. If, however, there is a real union at the nodes it is perfectly probable that parts of the two may be interchanged. For instance, if we take the case of a single twist, one end of chromosome *A* may be united with the other end of chromosome *B*, on the one side of the figure, with the reverse relation, on the other side. A factor located near one end of a chromosome is thus separated from one located near the other end. It is obvious that the nearer together two factors are within a chromosome the less chance there is that the crossing over of a twist will come between them. The percentage of such separation of characters in experimental breeding may then be taken as a measure of the distance apart of the factors in the chromosome assuming that the "twistability" of the chromosome is the same at all points. It is further natural to assume that the chromomeres are the seats of these separate factors.

It has already been mentioned that the percentage of separation between *a* and *c* tends to be somewhat less than the sum of the percentages between *a* and *b* and between *b* and *c*. This may be explained on the supposition that two twists sometimes take place between the more widely separated points and the result of two twists is the same as that of no twist as far as the factors in question are concerned. The percentage is therefore decreased for the greater distances. That such double crossing over occurs has been proved in other ways.

The fact of twisting of chromosomes has actually been observed in a number of cases, but the behavior of the chromomeres is hard to make out with any degree of certainty because they are near the limit of visibility even under the highest powers of the microscope. It is impossible, therefore, at present, to confirm by actual observation of the hereditary substance the hypothesis of exchange of material between the chromosomes in the manner just described. On the whole, the general evidence is favorable to the view, but there are still a number of difficulties. One of these has to do with the fact that crossing over takes place only in the female.

As far as the sex linked characters are concerned there is no difficulty, because in the male the sex chromosome either has no mate or has one with which no crossing over can occur. It is only in the female that crossing over is possible and the cytological evidence, therefore, is in agreement with the data of experimental breeding. With regard to the characters that

are not sex linked, there is, however, no satisfactory cytological explanation of the difference between male and female. A careful study is now being made by several workers particularly of the more difficult female material, and it is to be hoped that some definite conclusion may be reached on this important point.

Castle has recently attempted to show that a closer approximation to the data of percentage relations may be obtained by supposing that the factors do not have a strict linear arrangement. The hypothesis has also to meet difficulties due to the fact that the percentage of crossing over may be changed in various ways, though none of these changes affects the linear arrangement.

On the whole, the chromomere hypothesis still lacks some important elements before it can compare with the chromosome hypothesis in degree of demonstration. It has, however, already led to a number of very important discoveries regarding the method of inheritance and can therefore be said to have justified itself.

CONCLUSION

By correlating the data from experimental breeding with those from the microscopical examination of the germ cells, the biologist has been able to demonstrate the existence of a mechanism which explains many things about the manner in which characters are transmitted from generation to generation. To a large extent, the model is based upon the action of parts actually visible and clear to all observers. As the limit of visibility under the highest powers of the microscope is approached, as in the case of the chromomeres, there is however, a difference of opinion as to the facts. The imagination then comes into play and it may be that some of the structures figured are purely creatures of the imagination, just as the mannikins of the seventeenth-century observers were. This probability, however, does not invalidate the clearly demonstrated features of the model.

Having this model in mind, the biologist can plan manipulations similar to those which he would practise upon a machine large enough for the parts to be handled directly. A very great many discoveries of importance in the field of heredity have been the results of such imaginary manipulations.

But the biologist is not content to stop with the visible elements of his model. The cell, the nucleus and cytoplasm, the chromosomes and perhaps the chromomeres, are definite parts of a mechanical model that works in practise. But why do the

chromomeres act as they do? Why is one different in its action from others? The biologist now becomes a philosopher. He tries to picture to himself further extensions of the model he has built so far, on the basis of demonstrable data. On the basis of past achievement he is inclined to believe that the chromomeres differ in their action because they differ in structure and related function. Therefore they are not the ultimate units of the structure. It is natural for him to try to connect them with the units of the physicist and chemist, the so-called chemical elements and the electrons. But the gap between his model and that of the physicist is still too great to enable him to make any considerable use of the latter. The method of procedure in the two cases is much the same, but perhaps the construction of the physicist is the more speculative one.

As I have said, when the biologist comes to the last demonstrable elements of his model he is inclined to suppose that in the future it may be indefinitely extended by the same method of procedure which he has previously pursued and that it may at some time be linked up with the units of the physicists. Curiously enough, several eminent physicists have strongly contested such a possibility. They seem much more inclined than are the biologists to put a limit upon such an extension and to assume the existence of non-material factors. Perhaps they do not realize what the biologist knows all too well, namely, the hopeless sterility in the past of all such ideas of non-material factors. The devising of non-material factors is an interesting mental exercise. Men have been busy with it from the earliest times. But there is no indication that any considerable advance in our knowledge of organisms has been obtained in that way. Of course this does not prove that the truth may not lie in that direction. Since the great majority of people find satisfaction in postulating such non-material forces in explanation of observed activities, it is perhaps well that the small minority who find some satisfaction in constructing their incomplete mechanical models will never be able to make their models complete. For no matter how far such models are extended they will always finally end up in units, which will furnish opportunity for the ever-ready remark, "Aha! there you have something which your model does not explain. Must you not assume a non-material factor to explain *its* action?" The only answer that can be given is the one already stated—that as time goes on more and more of the activities of living things can be pictured in terms of demonstrable mechanical models.